Use of an Antibody to Target Geldanamycin

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The promise of antibodies as mediators of effective targeted anticancer therapy is beginning to be fulfilled. In the past few years, two monoclonal antibodies (MAbs) have been approved for selective use by the U.S. Food and Drug Administration (FDA) (Rockville, MD) and incorporated into standard therapy, resulting in improved clinical responses and prolongation of life. The most dramatic clinical response has been to rituximab, which is directed against the CD-20 antigen on lymphoma cells (1). Herceptin against the HER2 receptor has proved to be effective in combination with chemotherapy for a subpopulation of breast cancer patients whose malignant cells express high levels of this receptor (2).

Since the discovery of methods for producing large quantities of MAbs by Kohler and Milstein in 1975 (3), investigators have explored the following three ways of using MAbs to treat cancer: 1) as mediators of immune cytotoxicity through activation of complement or by action of lymphocytes and macrophages, 2) as inhibitors of specific functions mediated by the targeted antigen, and 3) as carriers of cytotoxic molecules or radionuclides to cells bearing the relevant antigen. The article by Mandler et al. (4) in this issue of the Journal combines the second and third approaches.

HER2 belongs to the epidermal growth factor (EGF) family of receptors, which also includes HER3 and HER4. When activated by specific ligands, these molecules form homodimers and heterodimers that transmit biochemical signals through stimulation of intrinsic tyrosine kinase activity. The first MAb capable of blocking EGF receptor activity, MAB 225, was reported in 1983 (5). Inhibitory activity depended on the capacity of the antibody to prevent binding of the natural ligands, EGF or transforming growth factor-α (5). This property inhibited activation of receptor tyrosine kinase and thereby inhibited proliferation of cells bearing EGF receptors, which include nearly all epithelial and mesenchymal cells. In culture and in xenograft models, antibodies against the EGF receptor inhibited cell proliferation (6,7), especially when combined with chemotherapy (8–10). In phase I/II clinical trials, human: murine chimeric antibody C225 against the EGF receptor showed activity in combination with chemotherapy (11) and radiation therapy (12).

The first antibody with comparable growth inhibitory properties against the HER2 receptor targeted the rat neu receptor, which is constitutively activated by a single point mutation in the transmembrane region (13,14). Subsequently, Genentech produced MAb 4D5, which became Herceptin in its humanized form (15,16). Herceptin can actually transiently stimulate activation of the HER2 receptor’s intrinsic tyrosine kinase, presumably by the unnatural mechanism of forming HER2–HER2 homodimers (17). This is followed by the internalization and catabolism of receptors, which is believed to be the most likely mechanism of action for these MAbs in inhibiting tyrosine kinase activity and cell proliferation. There is strong clinical evidence that cancer cells expressing high levels of HER2 are resistant to chemotherapy (18,19). A variety of studies (20–22) have demonstrated that the sensitivity of cancer cells growing in culture and in human tumor xenografts to chemotherapy with cisplatin, paclitaxel, or doxorubicin is increased by interventions that reduce HER2 levels and activity.

A phase II clinical trial with Herceptin demonstrated a 12% response rate (four partial responses and one complete response) in 43 previously treated patients with advanced metastatic breast cancer. This study (23) provided “proof of concept” that antireceptor treatment can produce clinical responses. A larger study (24) has confirmed these results. A randomized clinical trial demonstrated that the addition of Herceptin to chemotherapy with the combination of doxorubicin and cyclophosphamide or with paclitaxel resulted in an increased response rate of 62% compared with a response rate of 36% for patients with metastatic breast cancer receiving chemotherapy alone (25). At a median follow-up of 25 months, an overall survival advantage for added Herceptin was seen with the combination of doxorubicin and cyclophosphamide (33.4 versus 24.5 months, respectively) and with paclitaxel (22.1 versus 18.4 months, respectively) (2). Unfortunately, in the group treated with Herceptin, doxorubicin, and cyclophosphamide, class III/IV cardiac dysfunction reached 19% (2). The FDA approved administration of Herceptin with paclitaxel for advanced breast cancer in 1998. Currently, there are many trials exploring the use of Herceptin plus chemotherapy in earlier stages of breast cancer and against other types of cancer. To date, responses appear to be confined to patients whose tumors express high levels of HER2, which include only 25% of patients with breast cancer and some patients with lung or gastric cancer.

As pointed out in the article by Mandler et al. (4), these data describing modest increases in survival rates are very encouraging, but, at present, the utility of Herceptin is limited to patients with tumors that highly overexpress HER2 (18).

The ansamycin group of compounds, including herbamycin A and geldanamycin, has shown strong antitumor activity against human cancer cells in culture and in xenografts. Until recently, these agents were not being studied in clinical trials because of severe toxicity in vivo. Mechanisms that may explain the activity of geldanamycin have accumulated over the past 5 years. Originally, it was found that incubation of cells with ansamycins resulted in reduced activity of a variety of tyrosine kinases, including transmembrane receptors, such as the EGF receptor, HER2, and the insulin-like growth factor receptor, as well as intracellular tyrosine kinases, such as src and fyn [reviewed in (26)].

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Although it is possible that the ansamycins could be exerting direct inhibitory effects against kinases such as HER2, solid evidence points to an interesting mechanism of action involving polyubiquitination and proteasomal degradation of these kinase molecules (27,28). This appears to result from binding of heatshock protein 90, which disrupts its association with kinase molecules, and thus its chaperone activity, and leads to the ubiquitination and degradation of these kinases. In the case of HER2, receptor depletion seems to be caused by binding of the ansamycin to the glucose-regulatory protein 94, which is the endoplasmic reticulum homologue of heat shock protein 90 (29).

Other effects of ansamycins on cells that are unrelated to tyrosine kinase inhibition have been reported. They include inhibition of DNA polymerase activation (30), modulation of nuclear factor κ B (31), and destabilization of mutated p53 (32). Thus, it is possible that the cytotoxicity of these compounds may be related to functions other than ubiquitination.

Mandler et al. (4) have taken the bold step of attempting to deliver geldanamycin to one of its best characterized targets, HER2, by coupling geldanamycin to an anti-HER2 MAb. They reasoned that this might selectively enhance the capacity of the antibody to reduce expression of HER2, while avoiding the severe general cytotoxicity of geldanamycin. The project required chemical alteration of geldanamycin by the addition of a linker that enabled them to produce an immunoconjugate of this derivative bound to the anti-HER2 MAb e21. The modified geldanamycin that they created is 17-(3-aminopropylamino)geldanamycin (17-APA-GA), which they demonstrated could retain nearly complete activity.

With this accomplished, Mandler et al. demonstrated the capacity of the geldanamycin : e21 immunotoxin to target HER2-bearing tumor cells and to selectively reduce the levels of HER2 expression. Modified geldanamycin alone (at 50% inhibition concentrations [IC50]) or MAb alone reduced HER2 levels by 20% of control values, whereas equimolar concentrations of modified geldanamycin conjugated to e21 MAb reduced HER2 levels by 86%.

In parallel, the geldanamycin : e21 immunotoxin reduced [3H]thymidine incorporation at 24 hours of culture with an IC50 (based on molar concentration of geldanamycin) of 0.58 μM, compared with an IC50 of 0.18 μM for the unconjugated 17-APA-GA. Thus, Mandler et al. have successfully linked geldanamycin to a targeting molecule, with retention of its activity at concentrations nearly comparable to those of unconjugated geldanamycin. In contrast, MAb e21 at concentrations as high as 11 μM could reduce [3H]thymidine incorporation by only 20%.

Experiments also demonstrated that growth-inhibitory concentrations of geldanamycin can be delivered selectively to cells bearing HER2 receptors by the immunoconjugate since cells lacking HER2 expression were unaffected. Because treatment of cells with free geldanamycin at comparable concentrations could produce comparable growth inhibition, it is clear that conjugating geldanamycin to MAb protected cells from its cytotoxic effects unless they expressed high levels of HER2. Furthermore, a related anti-HER2 MAb, AE1, which is poorly internalized and does not alter tumor xenograft growth, did not acquire enhanced capacity to inhibit [3H]thymidine incorporation when conjugated with geldanamycin, underscoring the critical importance of internalization of geldanamycin into the cell (through action of the antibody) where it can bind to heat shock proteins or other targets.

These interesting results stimulate a number of questions that must now be addressed, if the geldanamycin : e21 immunotoxin is to move forward into clinical trials. The only measure of antiproliferative effects was [3H]thymidine incorporation at 24 hours. Studies examining effects on the cell number over a period of days, accompanied by measurement of cell death and apoptosis, are now in order. The immunotoxin must be tested against human tumor xenografts, along with appropriate controls, to examine efficacy and potential toxicity. Because MAb e21 presumably does not bind to murine receptors, toxicity will be difficult to measure.

The mechanisms explaining the antiproliferative activity induced by the immunotoxin also require further study. In these experiments, the inhibitory concentrations of geldanamycin that arrived inside the cell, whether delivered as a geldanamycin : e21 immunotoxin or as free geldanamycin, were nearly comparable. Although attachment to MAb e21 forces close proximity of geldanamycin to HER2 molecules in the cell, it is quite possible that the geldanamycin molecules could displace heat shock proteins from other critical proteins besides HER2, so that they too would become ubiquitinated and targeted for proteosomal degradation. There are also other possible mechanisms of action of geldanamycin conjugated with e21 besides these involving heat shock proteins (see above), and these need to be considered.

Conjugation of geldanamycin to an MAb builds on nearly two decades of research attempting to use MAbs to target cancer cells with specific toxins, such as modified ricin and saponins from plants and pseudomonas exotoxin and diphtheria toxin from bacteria. It has been a great challenge to devise optimal coupling mechanisms between MAbs and toxins. Modifications have been required to avoid nonspecific uptake in the liver and the reticuloendothelial system and to attempt to facilitate internalization into the optimal intracellular compartment (33). Several clinical trials with immunotoxins have documented antitumor activity in humans [reviewed in (33)]. However, serious clinical problems remain to be solved, especially the immunogenicity of toxins and nonspecific toxic effects such as the vascular leak syndrome.

Mandler et al. have taken the tack of conjugating a highly cytotoxic antibiotic molecule, geldanamycin, to an MAb. The reported observations give strong support to the conclusion that an active derivative of geldanamycin with potent cytotoxicity can be delivered selectively into HER2-bearing cells by conjugation to an anti-HER2 MAb that undergoes internalization. Further experiments will be required to determine whether this approach will be able to bypass some of the pitfalls that have occurred when promising immunotoxins were moved from studies in cell culture into in vivo models and, ultimately, into clinical trials.

REFERENCES


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**NOTE**

Editor’s note: J. Mendelsohn holds stock options and is on the Board of ImmunClone, New York, NY, which is conducting clinical trials with monoclonal antibody C225 against epidermal growth factor receptors.